

REPLACEMENT SHEET 1 / 9

Construct of Feline Thyrotropin-beta Subunit with First Intron

1 30
5'(GAA TTC)ATG ACT GCT ATC TAC CTG ATG TCC GTG CTT
met thr ala ile tyr leu met ser val leu

31 75
TTT GGC CTG GCA TGT GGA CAA GCG ATG TCT TTT TGT TTT CCA ACT
phe gly leu ala cys gly gln ala met ser phe cys phe pro thr
76 120
GAG TAT ATG ATG CAT GTC GAA AGG AAA GAG TGT GCT TAT TGC CTA
glu cys met met his val glu arg lys glu cys ala tyr cys leu
121 162
ACC ATC AAC ACC ACC ATC TGT GCT GGA TAT TGT ATG ACA CGG
thr ile asn thr thr ile cys ala gly tyr cys met thr arg

Intron 1
163 GTATGTAGTTCATCTCACTTCTTTAGCTGAAAATTAGATAAACCTAGACT
CAGTCCATTTCTATCCAGAAAGGAAATGAGATAAATCACACCTCATTTACAGACCTAAC
GGTCAITGGCTCCTTAGAGGTAGAGTCCCTAGGTTATAATATACGGACCTATCCATACAG
TTGGTACAGATAATTTTACAATAGTTTACTCCCAAAGTTTATTAAACCTTATCTTGTTCC
CACGATCAAGGATAAAAGAGAGGTGTGTGTATGTCAITTTTTTTTGTCTCTATAGGATT
CAGTGTGGATATGCTGAATTGGTATTGGGGAATGGGACTAAGGAATCCTCCCCAGTCTTA
TTTGATCTATGGGATGTAAGCGAATTAACATTTTGCTTCTCTGTGCTTCCCTCAG

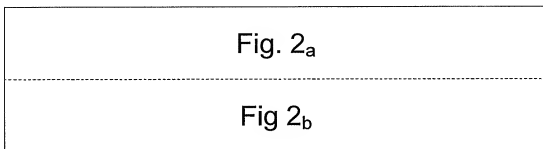
580 625
581 670
GAT ATC AAT GGC AAA CTG TTT CTT CCC AAA TAT GCT CTG TCC CAA
asp ile asn gly lys leu phe leu pro lys tyr ala leu ser gln
626 715
GAT GTT TGC ACC TAC AGA GAC TTC CTG TAC AAG ACT GTA GAA ATA
asp val cys thr tyr arg asp phe leu tyr lys thr val glu ile
671 760
CCA GGA TGC CCA CAC CAT GTT ACT CCC TAT TTC TCC TAC CCG GTA
pro gly cys pro his his val thr pro tyr phe ser tyr pro val
716 805
GCT GTA AGC TGT AAA TGT GGC AAG TGT AAT ACT GAC TAT AGC GAC
ala val ser cys lys cys gly lys cys asn thr asp tyr ser asp
761 835
TGC ATA CAT GAG GCC ATC AAG ACA AAT GAT TGT ACC AAA CCC CAG
cys ile his glu ala ile lys thr asn asp cys thr lys pro gln
806
AAG TCC GAT GTG GTA GGA GTT TCT ATC TAA (GCGGCCGC₍₄₎)(AT)₅-3'
lys ser asp val val gly val ser ile stop

() denotes the Eco RI restriction sites
Bold denotes signal sequence
Bold/italic denotes the intron 1 sequence

Fig. 1

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Feline Thyrotropin Alpha Subunit Construct



Bold denotes 24 amino acid unexpressed signal sequence as per structure in other species
Bold italics denotes sequence upstream from expressed but not secreted signal sequence;
only reported in equine; whether it is expressed is not clear.

() denotes Eco R1 restriction site from TOPO Blunt vector

Underlined denotes additional sequence from TOPO Blunt vector

((1)) denotes Factor XA site

((2)) denotes Flag tag

((3)) denotes stop codon

((4)) denotes NotI restriction enzyme site

()₅ denotes extra bases needed for restriction enzyme to work

Fig. 2

REPLACEMENT SHEET 3a / 9

(GAATTC) GCCCTT

1 45
AGT TAC TGA GAA ATC ACA AGA CGA AGC CAA AAT CCC TCT TCA GAT
 ser tyr OPA glu ile thr arg arg ser gln asn pro ser ser asp
 46 90
CCA CGG TCA ACT GCC CTG ATC ACA TCC TGC AAA AAG TCC GGA GGA
 pro arg ser thr ala leu ile thr ser cys lys lys ser gly gly
 91 135
AGG AGA GCC ATG GAT TAC TAC AGA AAA TAT GCA GCT GTC ATT CTG
 arg arg ala met asp tyr tyr arg lys tyr ala ala val ile leu
 136 180
GCC ATA CTC TCT GTG TTT CTG CAT ATT CTC CAT TCT TTT CCT GAT
 ala ile leu ser val phe leu his ile leu his ser phe pro asp
 181 225
GGA GAG TTT ACA ATG CAG GGG TGC CCA GAA TGC AAG CTA AAG GAA
 gly glu phe thr met gln gly cys pro glu cys lys leu lys glu
 226 270
AAC AAA TAC TTC TCC AAG TTG GGT GCC CCA ATT TAT CAA TGC ATG
 Asn lys tyr phe ser lys leu gly ala pro ile tyr gln cys met
 271 315
GGC TGC TGC TTC TCC AGA GCA TAC CCC ACT CCA GCA AGG TCC AAG
 gly cys cys phe ser arg ala tyr pro thr pro ala arg ser lys
 316 360
AAG ACA ATG TTG GTC CCA AAG AAC ATC ACC TCA GAA GCC ACA TGC
 lys thr met leu val pro lys asn ile thr ser glu ala thr cys
 361 405
TGT GTG GCC AAA GCC TTT ACC AAG GCC ACG GTA ATG GGA AAT GCC
 cys val ala lys ala phe thr lys ala thr val met gly asn ala

Fig. 2_a

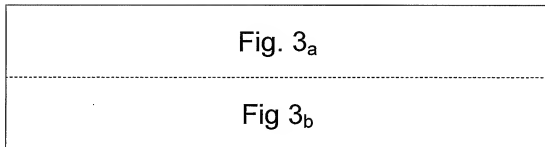
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406 450
 AAA GTG GAG AAT CAC ACA GAG TGC CAC TGC AGC ACT TGC TAT CAC
 lys val glu asn his thr glu cys his cys ser thr cys tyr his
 451 459 492
 CAC AAG ATT (ATC GAA GGT CGT₍₁₎)(GAC TAC AAG GAC GAT GAC GAT
 his lys ile ile glu gly arg asp tyr lys asp asp asp asp
 493 495 510
 AAG₍₂₎ (TAA₍₃₎) (GCGGCCGC₍₄₎)(TATG)₅ 3'
 lys

Fig. 2_b

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Yoked Feline Thyrotropin



- () denotes the Eco RI restriction sites
Bold denotes signal sequence
Bold italics denotes intron 1 sequence 1=Factor XA site
((1)) denotes Factor XA site
((2)) denotes Flag tag
((3)) denotes stop codon
((4)) denotes NotI restriction enzyme site
()₅ denotes extra bases needed for restriction enzyme to work

Fig. 3

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1 30
 5'(GAA TTC)ATG ACT GCT ATC TAC CTG ATG TCC GTG CTT
 met thr ala ile tyr leu met ser val leu

31 75
 TTT GGC CTG GCA TGT GGA CAA GCG ATG TCT TTT TGT TTT CCA ACT
 phe gly leu ala cys gly gln ala met ser phe cys phe pro thr

76 120
 GAG TAT ATG ATG CAT GTC GAA AGG AAA GAG TGT GCT TAT TGC CTA
 glu cys met met his val glu arg lys glu cys ala tyr cys leu

121 162
 ACC ATC AAC ACC ACC ATC TGT GCT GGA TAT TGT ATG ACA CGG
 thr ile asn thr thr ile cys ala gly tyr cys met thr arg

Intron 1

163 GTATGTAGTTCATCTCACTTCTTTAGCTGAAAAATTAGATAAACCTAGACT
 CAGTCCATTCTATCCAGAAAGGAAATGAGATAAATCACAACCTCATTTCACAG
 ACCTAACGGTCAITGGCTCCTTAGAGGTAGAGTCCCTAGGTTATAATATACGGA
 CCTACTCCATACAGTTGGTACAGATAATTTTACAATAGTTTTACTCCCAAAGTT
 TATTTAAACCTTATCTTGTGCCACGATCAAGGATAAAAAGAGAGGTGTGTGTGT
 ATGTCATTTTTTTTGTCTCTATAGGATTCAAGTGTGGATATGCTGAATTGGTATT
 GGGGAATGGGACTAAGGAATCCTCCCCAGTCCTATTTGTATCTATGCGGATGT
 AAGCGAATTAACATTTTGCTTCCTCTTCTGTGCTTCCCTCAG 580

581 625
 GAT ATC AAT GGC AAA CTG TTT CTT CCC AAA TAT GCT CTG TCC CAA
 asp ile asn gly lys leu phe leu pro lys tyr ala leu ser gln

626 670
 GAT GTT TGC ACC TAC AGA GAC TTC CTG TAC AAG ACT GTA GAA ATA
 asp val cys thr tyr arg asp phe leu tyr lys thr val glu ile

671 715
 CCA GGA TGC CCA CAC CAT GTT ACT CCC TAT TTC TCC TAC CCG GTA
 pro gly cys pro his his val thr pro tyr phe ser tyr pro val

716 760
 GCT GTA AGC TGT AAA TGT GGC AAG TGT AAT ACT GAC TAT AGC GAC
 ala val ser cys lys cys gly lys cys asn thr asp tyr ser asp

761 805
 TGC ATA CAT GAG GCC ATC AAG ACA AAT GAT TGT ACC AAA CCC CAG
 cys ile his glu ala ile lys thr asn asp cys thr lys pro gln

806 beta-specific primer sequence * CTP linker 850
 AAG TCC GAT GTG GTA GGA GTT TCT ATG CAG GAC TCC TCT TCC TCA
 lys ser asp val val gly val ser ile gln asp ser ser ser ser

851 CTP linker 892
 AAG GCC CCT TCC GCC AGC CTT CCA AGC CCA ACG CGT CTC CCG
 lys ala pro ser ala ser leu pro ser pro thr arg leu pro

* reverse complement in construct

Afl III ligation site

Fig. 3a

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893	CTP linker	alpha-specific primer sequence	937	*
GGG CCC TCG GAC ACC CCG ATC CTC CCA CAA		TTT CCT GAT GGA GAG		
gly	pro	ser	asp	thr
pro	ile	ile	pro	gln
phe	pro	asp	gly	glu
938			977	
TTT ACA ATG CAG GGG TGC CCA GAA TGC AAG CTA AAG GAA				
phe	thr	met	gln	gly
cys	pro	glu	cys	lys
leu	lys	glu		
978			1022	
AAC AAA TAC TTC TCC AAG TTG GGT GCC CCA ATT TAT CAA TGC ATG				
Asn	lys	tyr	phe	ser
lys	leu	gly	ala	pro
ile	tyr	gln	cys	met
1023			1067	
GGC TGC TGC TTC TCC AGA GCA TAC CCC ACT CCA GCA AGG TCC AAG				
gly	cys	cys	phe	ser
arg	ala	tyr	pro	thr
pro	ala	arg	ser	lys
1068			1112	
AAG ACA ATG TTG GTC CCA AAG AAC ATC ACC TCA GAA GCC ACA TGC				
lys	thr	met	leu	val
pro	lys	asn	ile	thr
ser	glu	ala	thr	cys
1113			1157	
TGT GTG GCC AAA GCC TTT ACC AAG GCC ACG GTA ATG GGA AAT GCC				
cys	val	ala	lys	ala
phe	thr	lys	ala	thr
val	met	gly	asn	ala
1158			1202	
AAA GTG GAG AAT CAC ACA GAG TGC CAC TGC AGC ACT TGC TAT CAC				
lys	val	glu	asn	his
thr	glu	cys	his	cys
ser	thr	cys	tyr	his
1203	1211			
CAC AAG ATT (ATC GAA GGT CGT ₍₁₎)(GAC TAC AAG GAC GAT GAC GAT				
his	lys	ile	ile	glu
gly	arg	asp	tyr	lys
asp	asp	asp	asp	asp
1245	1247		1262	
AAG ₍₂₎ (TAA ₍₃₎) (GCGGCCGC ₍₄₎)(TATG) ₅ 3'				
lys				
	*	as written		

Fig. 3_b

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PEAK Expression Vector (with yoked fTSH)

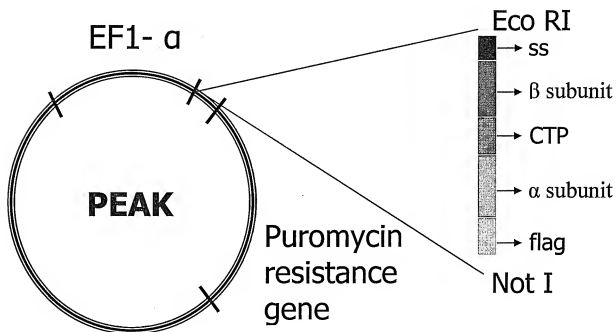


Fig. 4

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Fig. 5a

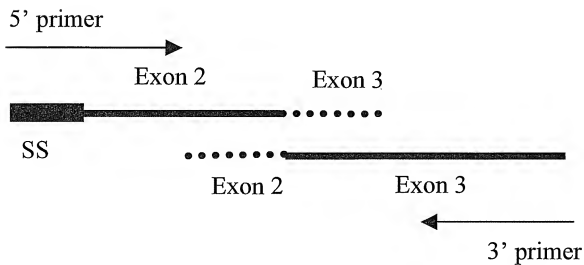


Fig. 5b

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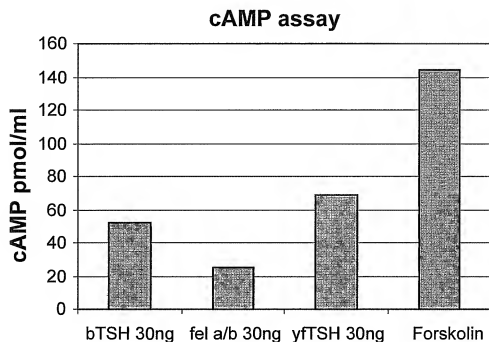


Fig. 6a

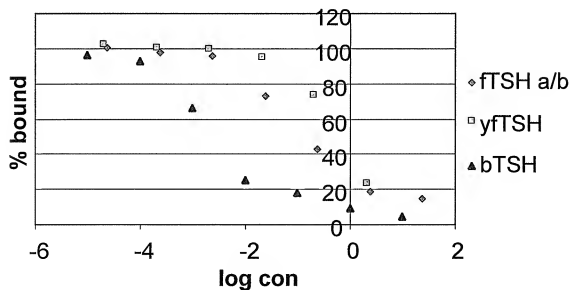


Fig. 6b